Comparative Study of the Inhibitory Activities of *Ocimum gratissimum* and *Nepeta cataria* against *Salmonella Enterica* Serovar Typhi and their Larvicidal Effect against *Anopheles Gambiae*

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ABSTRACT

This study was carried out to evaluate the antibacterial and larvicidal activity of Ocimum gratissimum and Nepeta cataria against Salmonella enterica serovar Typhi (S. Typhi) and Anopheles gambiae. S. Typhi was isolated from raw egg using Salmonella Shigella Agar (SSA), characterized and identified using Gram staining, colonial description and biochemical characteristics. The phytochemical constituents of the leaves extracts were determined quantitatively using spectrophotometric method. The antibacterial activity was carried out using agar-well diffusion method. Tube dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using double-fold serial dilution at concentration 25mg/ml to 400mg/ml. The larvicidal activity of the leaves extracts was carried out by subjecting A. gambiae larvae to varied concentration of the leaves extracts using in vitro assay at room temperature for 72 h. The phytochemical analysis of the leaves extracts revealed the presence of tannins, saponins, alkaloid, flavonoids, steroids, glycosides, phenolics and essential oil. The antibacterial activities of the leaves extracts showed that ethanol and aqueous extracts of N. cataria (16.30mm, 8.30mm) exhibited slightly higher activity than O. gratissimum (15.70mm,7.67 mm) and their activities differed significantly ($p \leq 0.05$) from that of ciprofloxacin (21.30 mm). The result of MICs and MBCs of the leaves extracts of N. cataria and O. gratissimum showed that the N. cataria leaf extract exhibited more inhibitory activities against the tested organism. The larvicidal activities of the leaves extracts at doses (0.1ml and 0.2ml) showed that O. gratissimum (40%, 46.67%) differed significantly by ($p \leq 0.05$) from N. cataria (33.3%, 46.67%) at their lower doses. The result of LT_{50} and LT_{90} of O. gratissimum (70,116) and N. cataria (71,117) revealed their pronounced larvicidal activities against A. gambiae. This study suggests that O. gratissimum and N. cataria leaves extracts could be used as alternative therapy for typhoid fever, and as pesticides for A. gambiae larvae.

Key words: Phytochemical, Antibacterial, Larvicidal, Typhoid Fever and Pesticide

INTRODUCTION

The occurrence of malaria and typhoid fever in the tropics, mainly in the developing nations of the world particularly in Africa remains a major concern. Malaria remains the most complex and overwhelming health challenge facing the world mostly in the tropical and subtropical regions with about 300 to 500 million cases and over 2 to 3 million death per year (Iheukwumere, Nwachukwu, and Kanu, 2013). Although, preventable and treatable, but has been a cause of severe maternal and childhood morbidity and mortality. Beside health impact it is a major impediment to socio-economic development (Iheukwumere *et al.* 2013). In contrast to developed nations, typhoid fever remains an important cause of illness in the developing countries with highest endemicity in the south-eastAfrica and south-central Asia with low rate of disease in other developing countries. However, typhoid fever is a major cause of morbidity in many regions of the world with an estimate of 12 to 33 million cases occurring annually (Crum, 2003).

In most developing countries, 90% of death occurring annually is usually attributed to malaria which is caused by *Plasmodium falciparium* the most infectious of the four human malaria parasites (*Plasmodium falciparium, Plasmodium ovale, Plasmodium malariae Plasmodium vivax*) with an estimated number of 1.4 to 2.6 million deaths per year. It is often transmitted by a malaria vector *Anopheles gambiae* (Iheukwumere *et al*. 2013). Typhoid fever a global health problem with an approximately 17 million cases and 600,000 deaths occurring annually and is often caused by *S. enteric* serovars *Typhi* a pathogenic, motile, Gram negative rod shaped bacterium which is transmitted by the ingestion of feacally-contaminated food and water with an incubation period of 10 to 14 days. It occurs with symptoms such as low-grade fever, malaise, headache, constipation in infected individuals and sometimes spleen and liver enlargement rose spot in skin of the infected persons (Dada and Komolafe, 2013).

In order to combat these disease the use of combine therapy of synthetic drugs and insecticide such as fluoroquinolone (e.g. Ciprofloxacin, Ofloxacin) used as a drug of choice for the treatment of typhoid fever and insecticide used in the control of malaria. But the major impediment to this effective chemotherapy is the ever increasing number of resistance strains of *S. typhi* therefore imposing public health problem (Cabrera *et al*., 2004). According to Das, Goswani, and Rabha, (2007) stated that the repeated use of synthetic insecticide resulting in the development of resistance mosquito species, undesirable effect on non-target organisms and also environmental and human health concern. This problem coupled with the high cost of the insecticide and drugs have revived interest in exploiting disease control potential plants. In this regard an alternative arsenal drugs in terms of medicinal plant based mosquitocidal and antibactericidal agent with effectual mode of action is needed. The identification and the use of these indigenous medicinal plant is much more employed as it is readily available and economical for use in the control of disease (Cabrera *et al*., 2004).

O. gratissimum an herbaceous and *N. cataria* an herbaceous mint plant widely distributed has been found to contain certain phytochemical such as alkaloid, saponins, flavonoid etc. with antimicrobial properties and are stored in varying concentration in the plant cells and has been found useful in treatment of bacterial infection and also toxic secondary metabolites from the plants were extracted, tested and were found effective against mosquito larvae (Okigbo, Okeke and Madu, 2010). Although many herbs have been used to investigate several disease and a considerable number of studies have emphasized the research and development of herbal substance in the control of diseases by previous researchers (Tsao, Roman, Peterson and Coast, 2002; Sukurmar, 1991; Abubakar, 2009; Adebolu, Adeoye, and Oyetaye, 2005) but only a few work has been done to investigate the antimicrobial and lavicidal activity of these plants extract.In respect to this, the inhibitory and larvicidal activity of *O. gratissimum* and *N. cataria* against *S. Typhi* and *A. gambiae* were evaluated.

MATERIALS AND METHODS

Sample Collection: The fresh leaves of *Nepeta cataria* and *Ocimum gratissimum* were collected from Obodo family of Ndumereaku Village in Uli, Ihiala Local Government Area, Anambra State.

Figure 1: Whole part of *Nepeta cataria*

Figure 2: Whole part of *Ocimum gratissimum*

Preparation of Sample for Extraction: The leaves of *Nepeta cataria* and *Ocimum gratissimum* were plucked off and dried under shade at room temperature for 14 days. The dried samples were pulverized using manual blender weighed and kept ready for extraction of active ingredients (Nwobu, Uzochukwu, and Okoye, 2010).

Extraction Procedure: A 10g portion extracted by maceration in 200 ml of ethanol and water respectively for 3 day. The resulting extracts were subsequently filtered using Whatman No.1 filter paper. The extracts were evaporated to dryness at room temperature in a steady air current (Nwobu *et al.* 2010).

Preparation of Test Sample: In this study, concentration of 400 mg/ml of the extract was used to screen for the antimicrobial activity. This was done by using the modified method NCCLS (2000). Here, 2.5 g of the extract was dissolved in each of the extracting solvents.

Isolation and Identification of Test Organism: The test organism used in this work was collected from an egg (layer's egg). The egg shell were swabbed using 70% alcohol and perforated using sterile fork and the inner fluid (albumin) sample were collected using 2 ml syringe and the sample was shaken vigorously. The sample were plated on Salmonella Shigella agar (SSA) and then incubated at 37^0C for 24 h. The pure culture of the test organism was identified using (slide agglutination reaction), colonial description and biochemical reactions (Iheukwumere and Umedum, 2013).

Maintenance of Test Organisms: The isolated test organism was used for the antibacterial sensitivity testing. Prior to the test, the organism was subculture on nutrients agar plate at 37^0Cof 24 h. Then after 24 h, the culture was transferred into nutrient broth and incubated at 37^0C for 24 h (Iheukwumere and Umedum, 2013).

Sensitivity Testing Using Agar-Well Diffusion Method: This was carried out using the modified method of Iheukwumere and Umedum, (2013). Each labelled plate was uniformly inoculated with the test organism using pour plating method. A sterile cork borer of 5 mm diameter was used to make well on the medium. One tenth millilitre (0.1ml) of various concentrations of the extracts were dropped into each labelled well, and then incubated at 37^0C for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation. Ciprofloxacin (500mg/ml) was used as control.

Determination of Minimum Inhibitory Concentration (MIC): This was carried out using the modified method of Iheukwumere and Umedum, (2013). Here, various concentrations of the extract were obtained using double-fold serial dilution. Each dilution was assayed against the test organism using tube dilution method. One millilitre of the test organism was added into each dilution and incubated at 37^0C for 24 h. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. This was determined and recorded.

Determination of Minimum Bactericidal Concentration: This was determined using the modified method of Iheukwumere and Umedum, (2013). Here, equal volumes of various concentration of those tubes that did not produce any visible growth from MIC was plated on flesh sterile poured plated culture and incubated at 37^0C for 24 h. The lowest concentration of the extracts that killed the test organism was taken as the MBC.

Mosquito Larva: Mosquito larva of *Anopheles gambiae* were collected from High TECH parasitological laboratory at Port-Harcourt, Rivers State, and used in this study. Larvae were allowed to emerge in plastic containers filled with water. At the 11 instars stage, the larvae were transferred to large buckets (37 x 31 x 6cm). Larvae were fed on tetramin fish food and the water temperature was maintained at room temperature (28 ± 2°C) throughout larval development (Innocent, Cosam, Nicholas and Mainen, 2008).

Lavicidal assay: Lavicidal assay was carried out by exposing 15 late III or early IV instars larvae of *Anopheles gambiae* to various concentrations of the leaves extracts (ethanol extract and aqueous extracts). Different concentrations of 200, 100, 50, 25 and 12.5ppm of the leaves extracts were added in beakers containing the larvae in 50 ml of water sample solution and 0.1 ml of ethanol (water temperature 28 ± 2°C). The control experiment contained only 0.1ml of ethanol (blank). The test was triplicated from separately reared batches of larvae. The larvae were fed on fish food at 1 mg per beaker per day. The numbers of larvae dead were taken per 24 h for 3 days (Innocent *et al*, 2008). The experiment was repeated using different crude phytochemical extracted from the leaves extracts with the concentrations (200, 100, 50, 25 and 12.5 ppm). The numbers of larvae dead were also recorded after every 25 h at the same condition as above for 3 days.

Statistical Analysis: The results of the antibacterial activity of the replicates were expressed as mean ± standard deviation (SD) and the statistical analysis was done by the students' t test at 95% confidence level (Chao-Hsun, Yu-chun, Yu-fen and Ming-Hsian, 2010).The average mortality rate obtained from the lavicidal activity were subjected to probit analysis for calculating LT_{50} and LT_{90} statistical slope and the chi square values by adopting the method of Raddy, Karishma, Munthy and Jamil, (1992). The confidence limits were $P \leq 0.05$ or $P \leq 0.01$.

RESULTS

The qualitative phytochemical analysis of the leaves extracts of *Ocimum gratissimum* and *Nepeta cataria* were showed in tables 1. The analysis revealed the presences of alkaloid, tannins, saponin, phenolics, flavonoids, steroid and cardiac glycoside present in *Ocimum gratissimum* while alkaloid, tannins, saponin, phenolic and flavonoid found in *Nepeta cataria*. The presence of these phytochemical in the leaves could be responsible for their activity. The diameter zones of inhibition of the leaves extracts of *Ocimum gratissimum* and *Nepeta cataria* against *S. enteric* Typhi are shown in figures 3 and 4. The results showed that the extracts were effective against the tested organism. The ethanolic extracts showed more activity than the aqueous extracts and their activities differed significantly ($p>0.05$) from that of the ciprofloxacin (control). *Nepeta cataria* exhibited slightly higher activity than *Ocimum gratissimum* extracts. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Ocimum gratissimum* and *Nepeta cataria* leaves extracts are shown in figures 5a, 5b, 6a and 6b. The results of the study showed that the two leaves extracts exhibited similar inhibitory effect but only *Ocimum gratissimum* exhibited cidal activity.

The larvicidal activities of *Ocimum gratissimum* and *Nepeta cataria* aqueous leaves extracts are shown in table 5. The results showed that the leaves extracts were able to exhibit cidal activity against *Anopheles gambiae*. The larvicidal activities of the leaves extracts are dose dependent. The higher the dose, the higher the activity. *O. gratissimum* leaf extract showed similar activity with *N. cataria* after 72 h exposure at higher doses. The activity of *Ocimum gratissimum* differed significantly ($p< 0.05$) from that of *Nepeta cataria* at lower doses. The mortality rate of the leaves extracts of *Ocimum gratissimum* and *Nepeta cataria*, and their various LT_{50} and LT_{90} are shown in table 6. The results of the mortality rate of *O. gratissimum* are higher than that of *N. cataria* at lower doses (0.1ml). The time taken to kill 50% (LT_{50}) and 90% (LT_{90}) of the larvae by the leaves extracts were extrapolated using probit analysis as shown in table 6 and figures (70,116 and 71,117 at 0.1ml while at 0.2 ml were 66,106 and 65,108). This showed the potencies of the leaves extracts against the larvae.

Table 1: Phytochemical constituent of the leaves extract studied

Phytochemical	*Ocimum gratissimum* (%)	*Nepeta cataria* (%)
Alkaloids	9.74	5.12
Tannins	7.16	8.16
Saponins	2.94	1.28
Saponins	2.26	2.74
Flavonoids	3.87	3.61
Steroids	0.03	-
Cardiac glycosides	2.67	-
Essential oil	17.60	17.10

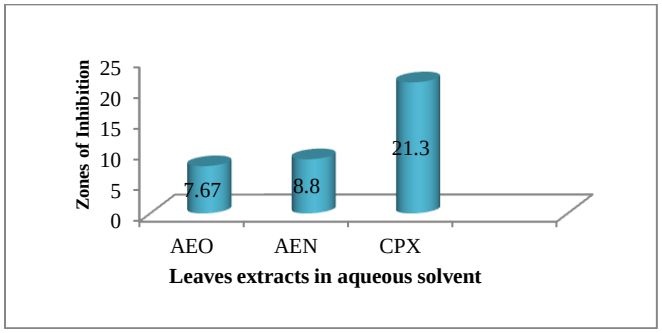

Figure 3: Diameter zones of inhibition of aqueous extracts *Nepeta cataria* And *Ocimum gratissimum* against the test organism
AEO = Aqueous Extract of *Ocimum gratissimum*, AEN = Aqueous Extract of *Nepeta cataria*, CPX = Ciprofloxacin

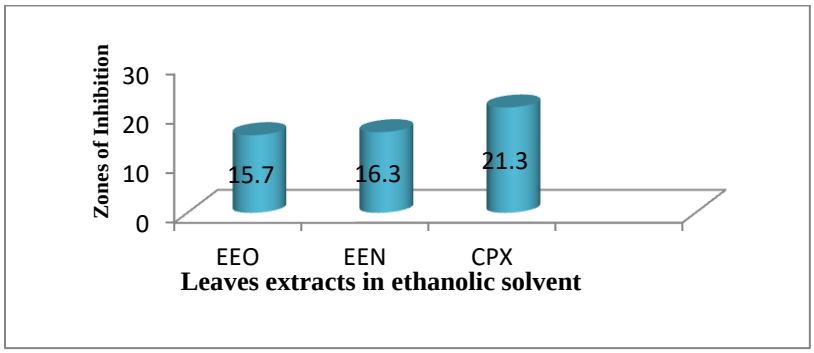

Figure 4: Diameter zones of inhibition of ethanol extract *N. cataria* and *O. gratissimum* against the test organism EEO = Ethanolic Extract of *Ocimum gratissimum*, EEN = Ethanolic Extract of *Nepeta cataria;*
CPX = Ciprofloxacin

Table 2: Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of the leaf extracts

Extacts (400mg/ml)	MIC (mg/ml)	MBC (mg/ml)
EEO	400	400
AEO	-	-
EEN	400	-
AEN	-	-
CPX	200	400

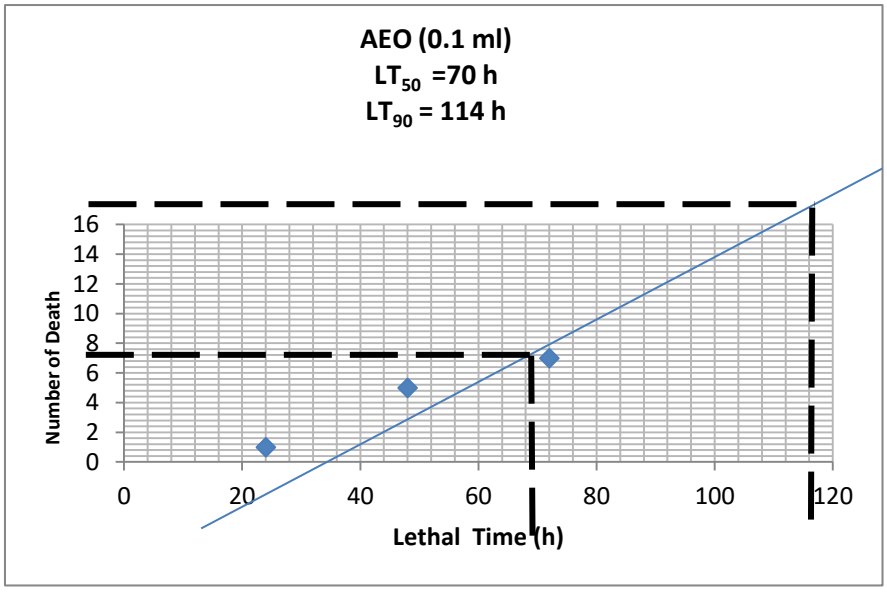

Figure 5a: Larvicidal activities of the leaves extract *O. gratissimum* at 0.1 ml

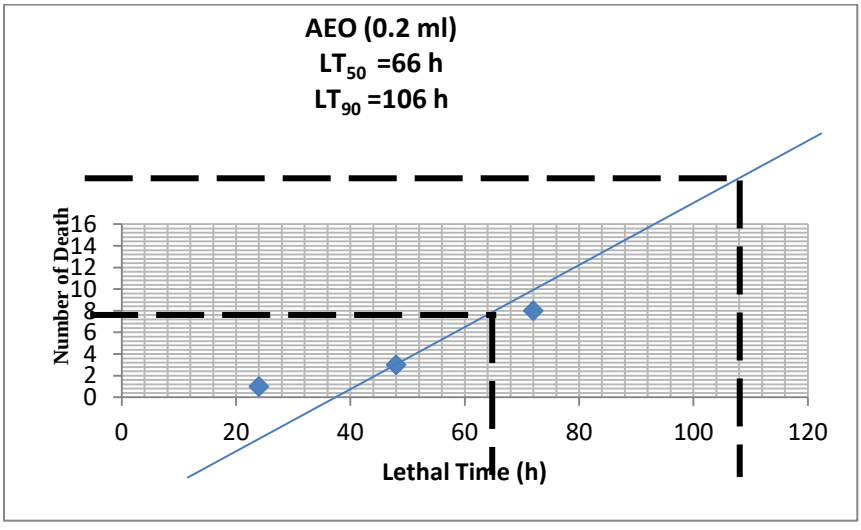

Figure 5b: Larvicidal activities of the leaves extract *O. gratissimum* at 0.2 ml

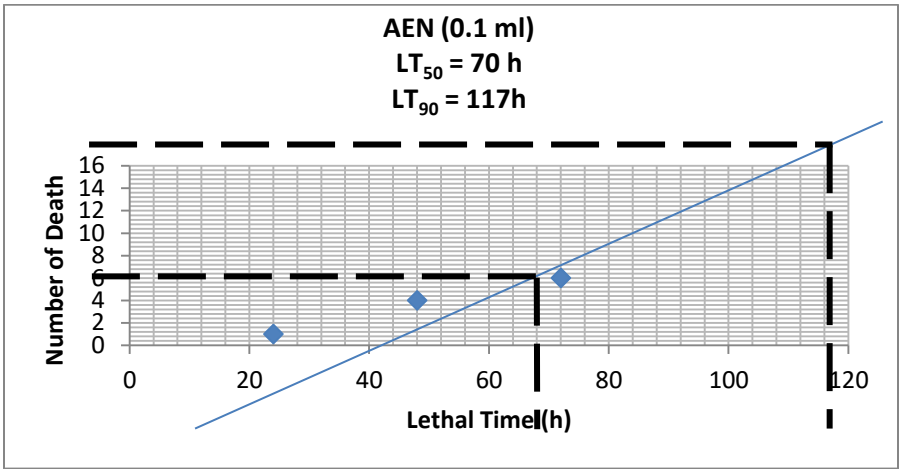

Figure 6a: Larvicidal activities of the leaves extract *N. cataria* at 0.1 ml

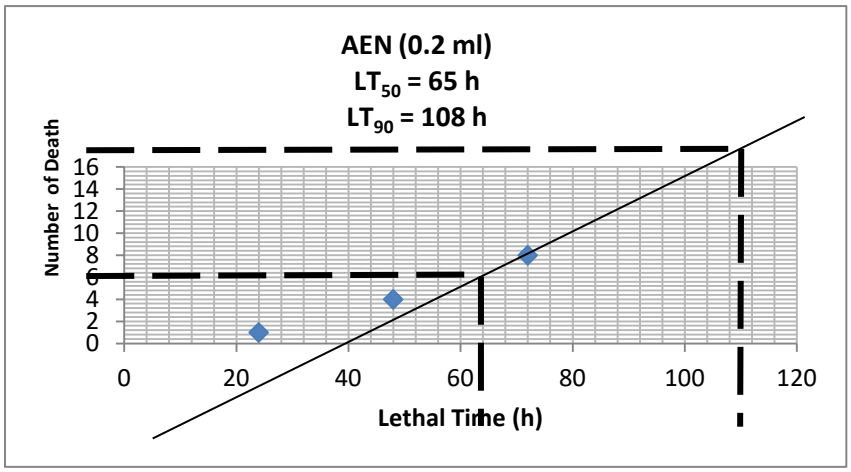

Figure 6b: Larvicidal activities of the leaves extract *N. cataria* at 0.2 ml

DISCUSSION

The spread of malaria and typhoid fever cause by *Anopheles gambiae* and *Salmonella enteric* serovarsTyphi remains the disease of major concern in the world with significant morbidity and mortality (Uneke, 2008). The repeated use of synthetic drug and insecticide in the control of diseases has resulted to the development of resistance species hereby causing environmental and public health problem (Odalo, Omalo, Angira, Njeru, Ndiege, and Hassanali, 2005). Thus, the use of plant extract has been found to be an effective way of controlling these diseases compared to the use of synthetic chemicals. As plant extract has several advantages overit (Oparaocha, 2008). In the present study the inhibitory and lavicidal effect of *Ocimum gratissimum* and *Nepeta cataria* against enteric pathogen *salmonella enteric* serovarsTyphi isolated from egg albumin and mosquito vector *Anopheles gambiae* were evaluated. The assay revealed clearly pronounced activity of the extracts on both the test organism and on the larvae.

The phytochemical analysis of the leaves extracts of *Ocimum gratissimum* and *Nepeta cataria* revealed alkaloid, flavonoid, saponins, tannins, steroid, glycosides and phenolics. The presences of these phytochemicals in the leaves extract may be responsible for their activity as described by many researchers (Cavalcanti, Morris, Lima, and Sanitaria, 2004; Okigbo *et al.* 2010; Bandh *et al.*, 2011; Sheu *et al.* 2012; Ofoegbu, Onyedineke, Nwokeji, Esie, and Isibor, 2013). Thus, some phytochemical work by intercalating with DNA of the organism (alkaloid), interferes with protein synthesis and disrupt cell membrane (e.g. saponins) while others interfere signal transduction pathway, metabolic processes, damage metabolic and cellular enzymes, disrupt proton motive force, electron flow,

coagulation of cell component and modulation of gene expression (Kotzekidou, Giannakidis, and Boulamatsis, 2008).

The result of the present study revealed the antibacterial properties of the ethanolic and aqueous extracts of the leaves *Ocimum gratissimum* and *Nepeta cataria* against *Salmonella enteric* serovarTyphi. The results revealed pronounced inhibition of the ethanolic and aqueous extracts of the leaves extracts against the tested organism. It was also observed that the activities of the ethanolic extracts were higher than aqueous extracts. This shows that the phytochemicals present has higher ability to dissolve in ethanol than aqueous solvent used in the study. This follows the result reported by Bandh *et al.* (2011). Although aqueous extract was a large amount of the leaves extract were produced but it exhibited lower effect on the test organism than the ethanol extract. Hence, the quantity of extracts produce does determine the rate of inhibition but the phytochemicals present.
Thus, the result of this research showed that ethanol has a higher ability to extract more and most of the phytochemicals in the leaves extracts under study. This may be due to the fact that ethanol is an organic solvent and most phytochemical constituent are organic in nature and is likely to dissolve in organic solvent than aqueous solvent. This follows the report by other researchers (Adebolu *et al.* 2005; Parakh, Jadeja and Chanda, 2005). These suggest that the organic solvent extraction was suitable for verification of the antimicrobial properties of the medicinal plant as this also is in line with report by Adebolu and Salau, (2005).

The result also revealed that *N. cataria* extracts inhibited more the test organism than *O. gratissimum*. These could due to the presences of more active phytochemicals such as tannins in *N. cataria* than in *O. gratissimum*. Tannins which are polyphenols with ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes as stated by Omojate, Enwa, Jewo and Eze, (2014). This, could be responsible for the pronounced inhibition exhibited by *N. cataria*. It also revealed that the leaves extracts showed relatively much inhibition on the organism *Salmonella enteric* Typhi. As this is line with the report by other researchers (Adebolu *et al.,* 2005; Parakh *et al.* 2005). The result of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the two leaves extracts revealed that ethanol and aqueous extracts showed reasonable antibacterial activity against the tested organism. The ethanolic and aqueous extract of the two extract showed similar activity but the activity of the ethanol extract of *Ocimum gratissimum* is slightly higher than in *Nepeta cataria*. This indicates that *S. enteric* serovarTyphi is more sensitive to the later than the former. Further research involving in vivo assay of the two leaves extract would be required to establish the relationship between the MIC and MBC obtained in this study and also the effective doses that should be administered in ethno-medical.

The result of the larvicidal activities of the leaves extracts of *O. gratissimum and N. cataria* showed the abilities of the extracts to kill the larvae of *Anopheles gambiae* a different dose. The larvicidal activities of these extracts may be attributed to the potent phytochemicals which have the ability to disrupt the cell membrane of the larvae and interferes with their protein synthesis. Similar reports were presented by other researchers (Sofowara, 1993; Adeowole, Oderinde, Banlade, Faparus and Oyede, 2012). The results of the present study revealed that *O. gratissimum* leaf extract was able to show higher cidal activity than *N. cataria* when applied at lower doses after 72 h exposure. The variation in activity at lower doses could be attributed to the variation in saponins which later became subdued due to increase in dosage. Saponins specifically work by disrupting the cell membrane and mitotuble spindles of the mosquito larvae (kotzekidon*et al.,* 2008). The results of the mortality rate, $LT_{50}$and LT_{90} of the leaves extracts showed that *O. gratissimum* leaf extract will take shorter time to kill greater percentage of the larvae than *N. cataria*. This could be as a result of more potent phytochemicals present in *O. gratissimum* than *N. cataria*. This is in line with similar observation made by some researchers (Cavalcanti *et al.* 2004; Okigbo *et al.,* 2010; Adeowole *et al.,* 2012) in their attempt to compare larvicidal activities of different leaves extracts.

CONCLUSION

The use of plant extract in the control of diseases has gained major role over the use synthetic agents. The result of the antibacterial and larvicidal assay of the leaves extract showed futuristic evidences for an effective antibactericidal and larvicidal activities against the test organism and mosquito larvae.

REFERENCES

Abubakar, E. M. (2009). Antibacterial activity of crude extracts of euphorbia hirta against some bacteria associated with enteric infection. *Journal of Medicinal Plant Resistance* **3**(7): 498–505.

Adebolu, T. T., Adeoye, O. O. and Oyetaye, V. O. (2011). Effect of galic (*Allium sativum*) on *Salmonella typhi* infection, gastrointestinal flora and heamatological parameters of albino rats. *African Journal of Biotechnology* **10** (35): 6804–6808.

Adewole, A., Oderinde, A., Banlade, O. Faparus, F. and Oyede, R. T. (2012). Larvicidal activities of three plants extracts of common wire weed (*Sida acuta*), catnip (*Nepeta cataria*) and neem (*Azadrichta indica*) against the larva of mosquito (*Anopheles gambiae*). *Academia Journal of medicinal plants* **1**(2): 37–40.

Bandh, A. S., Lone, A. B., Chrishti, Z. M., Kanti, N. A., Gonsii, A. B. and Saleem, S. (2011). Evaluation of anthelminthic and antimicrobial activity of the methanolic extracts of *Nepetacataria***4** (8): 29–135.

Cabarera, R., Luiz, J., Macro, F., Oliveira, I., Arrayo, M., Aladnena, A., Usera, M., De Anta, M.T., Gascon, J. and Vila, J. (2004). Mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing travellers'diarrhea. *Antimicrobial Agents of Chemotherapy* **48**(10): 3934–3939.

Cavalcanti, E. S. B., Morris, S. M., Lima, M. A. A. and Sanitaria, E. W. P. (2004). Larvicidal activity of essential oils from Brazilian plant against *Aedesaegyptic* (L). *Memorias De Institute Oscoaldo Cruz* **99**:541–544

Chao-Hsun, J., Yu-chun, H., Yu-fen, C. and Ming-Hsian, C. (2010). Foam properties, detergent abilities and long term preservative efficacy of the saponins from saponin adusmukorossi. *Journal of Food and Drug analysis* **18**:155–160.

Crum, N. F. (2003). Current trends in typhoid fever. *Current Gastroenterology* **5:**279–286.

Dada and Komolafe, O. I. (2013). In vitro evaluation of the inhibitory effect of *Ocimum gratissimum* against *Salmonella typhi*. *International Journal of Pharmaceutical and Biological Research* **4**(4): 126–129.

Das, N. G., Goswani, D. and Rabha, B. (2004). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *Journal of Vector Borne Diseases* **44**:145–148.

Iheukwumere, I. H. and Umedum, C. U. (2013). Effect of *Gossypium hirsutum* leaf extracts on Gram negative bacteria isolated from the cervix of females with unexplained infertility. *African Journal of Science* **14**(1): 3261–3270.

Iheukwumere, I., Nwachukwu, C. N., Kanu, M. A. (2013). Manifestation, management and diagnostic challenges of malaria and typhoid. Malaria *Chemotherapeutic Control Elimination* **2**:109–110.

Innocent, E., Cosam, C.J., Nicholas, K. G. and Mainen, J. M. (2008). Mosquito larvicidal constituent from lantana viburnoides. *Journal of Vector Borne* Disease **45**:240–244.

Kotzekidou, R. Giannakidis, P. and Boulamatsis, A. (2008). Antimicrobial activity of some plant extracts and essential oils against food borne pathogens in vitro and on the fate of inoculated pathogen in chocolate. *Food Science of Technology* **41**: 119–127.

Nwobu, R. A. U., Uzochukwu, I. C. and Okoye, E. I. (2010). Phytochemical analysis and antimicrobial activity of *Hyptissurveoleus*.*Medicinal Plant Phytochemicals Pharmacological Therapentices***1**:390–396.

Odalo, C., Mutua, A., Kindt, R., Jamnadass, R., Simons, A. (2005). Agrofores tree Database. A tree reference and selection guide version 4.0.

Odalo, J., Omalo, M. M., Angira, J., Njeru, P., Ndiege, I. and Hassanali, A. (2005). Repellency of essential oils of some plants from the kanyan coast against *Anopheles gambiae*. *ActaTropica,* **95**:210–218.

Ofoegbu, U. P., Onyedineke, E. N., Nwokeji, C., Esie, G. N. and Isibor, N. G. (2013). Laboratory evaluation ethanolic and methanolic extracts of *Ocimumgratissimum* against larva of *Anopheles gambiae*and non-target organism. *Muris Entomology and Zoology* **8**(1):185–190.

Okigbo, R. N., Okeke, J. J., and Madu, N. C. (2010). Larvicidal effects of *Azadirachtaindica*, *Ocimumgratissimum* and *Hyptissuaveotens*against mosquito larvae. *Journal of Agricultural Technology* **6** (4): 703–719.

Omojate, G. C., Enwa, F. O., Jewo, A. O. and Eze, C. O. (2014). Mechanism of antimicrobial actions of phytochemicals against enteric pathogen. AReview.Journal of Pharmaceutical, Chemical and biological sciences **2**(2):77–85.

Oparaocha, E. T. (2008). Preliminary study on mosquito repellant and mosquitocidal activities of *Ocimumgratissimum* (Liston) grown in eastern Nigeria. *Journal of Vector Borne Diseases* **44**(4):328–334.

Parekh, J., Jadeja, D., Chanda, S. (2005). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk. Journal of Biology* **29**:203–210.

Raddy, P. J., Karishma, D., Munthy, U. S. and Jamil, K. (1992). A microcomputer FORTRAN program for rapid determination of lethal concentration of biocides in mosquito control. *Cabios***8**:209–213.

Sheu, A., Umana, U. E., Timbuak, J.A., Hanman, Q., Lalymus, S. A. and Ikeyemba, D. T. (2012). Effect of aqueous ethanolic extracts of *Ocimumgratissimum* on the histology of spleen haematological indices of wister rats and its antimicrobial properties. *Asian Journal of Medicinal Sciences* **4**(4):130–133.

Sofowona, A. (2003). Medicinal plants and traditional medicine in Africa.Spectrum book cid, Ibadan. Pp 55–71.

Sulaurmar, K., Perich, M. J. and Boobar, L. R. (1991). Botanical derivative in mosquito control. *Journal of the American Mosquito Control Association* **7**:210–237.

Tsao, R., Roman, C. F. E., Peterson, C. J. and Coast, J. R. (2002). Plant growth regulatory effect and insecticidal activity of extracts of tree of heaven (Atlanthusaltissima). *BMC Ecology* **2**:1–18.

Uneke, C. J. (2008). Concurrent malaria and typhoid fever in the tropics. The diagnostic challenges and public health implication. *Journal of Vector Borne Diseases* **45**:135–142.